Recombinational repair in yeast: functional interactions between Rad51 and Rad54 proteins

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Rad51p is a eukaryotic homolog of RecA, the central homologous pairing and strand exchange protein in *Escherichia coli*. Rad54p belongs to the Swi2p/Snf2p family of DNA-stimulated ATPases. Both proteins are also important members of the *RAD52* group which controls recombinational DNA damage repair of double-strand breaks and other DNA lesions in *Saccharomyces cerevisiae*. Here we demonstrate by genetic, molecular and biochemical criteria that Rad51 and Rad54 proteins interact. Strikingly, overexpression of Rad54p can functionally suppress the UV and methyl methanesulfonate sensitivity caused by a deletion of the *RAD51* gene. However, no suppression was observed for the defects of *rad51* cells in the repair of γ-ray-induced DNA damage, mating type switching or spontaneous hetero-allelic recombination. This suppression is genetically dependent on the presence of two other members of the recombinational repair group, *RAD55* and *RAD57*. Our data provide compelling evidence that Rad51 and Rad54 proteins interact *in vivo* and that this interaction is functionally important for recombinational DNA damage repair. As both proteins are conserved throughout evolution from yeasts to humans, a similar protein–protein interaction may be expected in other organisms.
Keywords: DNA repair/*RAD51*/*RAD54*/recombination/ *S.cerevisiae*

Introduction

DNA repair is an essential part of the cellular response to DNA damage. Such damage occurs as a consequence of the cellular metabolism or by environmental exposure to chemical or physical agents. Accurate repair of DNA lesions avoids mutations and thereby ensures survival. Genetic analysis in *Saccharomyces cerevisiae* has identified mutations in nearly 100 genes, that affect DNA damage repair (reviewed in Friedberg *et al.*, 1995). Some of these genes have been assigned to epistasis groups that reflect an organization into DNA damage repair pathways (reviewed in Haynes and Kunz, 1981; Game, 1983). They include the nucleotide excision repair pathway (NER, *RAD3* group), the error prone/post-replication repair path-

way (*RAD6* group) and the recombinational repair pathway (*RAD52* group) (see Friedberg *et al.*, 1995).

DNA lesions like double-strand breaks (DSBs) that affect both strands of a double helix are particularly damaging. DSBs are the major genotoxic lesions induced by ionizing radiation, and the widespread use of this radiation in medical diagnosis and therapy requires a better understanding of the repair mechanisms for such lesions. In *S.cerevisiae*, such damage is repaired primarily by homologous recombination using the DNA sequence residing on the sister chromatid or homolog to restore the DSB in a way that is intrinsically error free (Resnick, 1976; Szostak *et al.*, 1983). Other pathways of DSB repair include single-strand annealing and end joining mechanisms that are intrinsically error prone (reviewed in Roth and Wilson, 1988; Haber, 1992). These pathways can be detected in *S.cerevisiae* (Fishman-Lobell *et al.*, 1992; Kramer *et al.*, 1994), but may be of greater relative importance in higher eukaryotes (for review, see Weaver, 1995; Wood, 1996). Mutations in the *RAD52* group genes result in pleiotropic defects in DNA damage repair including extreme sensitivity to ionizing radiation or other DSB-inducing agents and alkylating agents like methyl methanesulfonate (MMS) as well as sensitivity to UV irradiation (reviewed in Game, 1993; Friedberg *et al.*, 1995; Shinohara and Ogawa, 1995). Moreover, additional defects in various aspects of chromosome metabolism during vegetative growth and during meiosis have been described for some mutants, although not all genes have been analyzed systematically for all phenotypes (reviewed in Kleckner *et al.*, 1991; Petes *et al.*, 1991; Roeder, 1995).

The characterization of the *RAD52* group genes identified *RAD51*, *RAD52* and *RAD54* as the most important members based on the severity of the mutant phenotypes (Game, 1993). *RAD51* encodes a protein with sequence similarity to the central bacterial recombination protein RecA (Aboussekhra *et al.*, 1992; Basile *et al.*, 1992; Shinohara *et al.*, 1992). The protein forms protein–DNA filaments indistinguishable from RecA protein–DNA filaments (Ogawa *et al.*, 1993) and promotes homologous pairing and strand exchange *in vitro* (Sung, 1994; Sung and Robberson, 1995). Rad51 and Rad52 proteins interact as shown by genetic (Milne and Weaver, 1993; Schild, 1995), biochemical (Shinohara *et al.*, 1992) and molecular (Donovan *et al.*, 1994) experiments. Whereas the RecA paradigm (reviewed by West, 1992; Kowalczykowski and Eggleston, 1994) provides a framework for understanding the role of Rad51p, the function of Rad52p remains more mysterious. However, the interaction of Rad52p with Rad51p and with Rpa (Firmenich *et al.*, 1995), the eukaryotic single-stranded DNA-binding protein, suggests that Rad52 protein also acts directly on the DNA during recombinational repair.

The *RAD54* gene has been cloned (Calderon *et al.*, 1983) and sequenced (Emery *et al.*, 1991), and the predicted 898 amino acid Rad54 protein exhibits significant sequence homology to Swi2p/Snf2p. This homology includes seven sequence motifs that have been proposed to identify members of a family of putative DNA helicases (Gorbalenya and Koonin, 1993). The Snf2p/Swi2p family includes 28 eukaryotic and one prokaryotic member (Eisen *et al.*, 1995). The proteins function in transcriptional regulation (Snf2p/Swi2p, Mot1p, lodestar, brahma) and various DNA damage repair pathways including NER (Rad16p), post-replication repair (Rad5p), strand-specific repair (Rad26p, ERCC6) and recombinational repair (Rad54p). Biochemical analysis of Snf2p/Swi2p (reviewed in Peterson and Tamkun, 1995) failed to demonstrate helicase activity for the protein but identified a DNA-stimulated ATPase activity. Therefore, it is likely that Rad54p also acts directly on DNA, constituting, together with Rad51 and Rad52 proteins, a central component, critical for recombinational repair.

Recently, an astounding evolutionary conservation of the NER pathway between yeasts and humans has been established, making *S.cerevisiae* an attractive model system for this repair system (reviewed in Hoeijmakers, 1993). A similar situation might be true for the recombinational repair *RAD52* group, although the studies in higher eukaryotes are far from being complete. Already it appears that *RAD51* is conserved in most, if not all eukaryotes (Shinohara *et al.*, 1993; reviewed in Heyer, 1994), and homologs in Archaea have been identified recently (Sandler *et al.*, 1996). Moreover, genes with limited sequence homology to *RAD52* have been identified in higher eukaryotes (Bezzubova *et al.*, 1993; Bendixen *et al.*, 1994; Muris *et al.*, 1994). The third component of the putative core machinery in recombinational repair, Rad54p, is also conserved in structure and function, as the human homolog, *hHR54*, has been demonstrated as the first mammalian recombinational repair gene to complement a deletion in the corresponding *S.cerevisiae* gene (Kanaar *et al.*, 1996).

Here we show that the *S.cerevisiae* Rad51 and Rad54 proteins interact in the two-hybrid system and that an *in vivo* complex containing both proteins can be demonstrated biochemically by co-immunoprecipitation. Genetic experiments provide compelling evidence that this protein–protein interaction is significant in recombinational DNA damage repair. Most importantly, overexpression of Rad54p suppressed DNA damage repair-related phenotypes caused by a deletion in the *RAD51* gene. It appears, therefore, that this protein–protein interaction is important for the molecular mechanism of recombinational repair.

Results

Rad51p and Rad54p interact in the two-hybrid system

Recombinational repair is likely to be mediated by a multi-protein complex. Identifying the subunits of such a complex and their mutual interactions will be of great importance in understanding the molecular mechanism of this process. Using the two-hybrid system (Fields and Song, 1989; Gyuris *et al.*, 1993), we have identified an interaction between the Rad54 and Rad51 proteins of
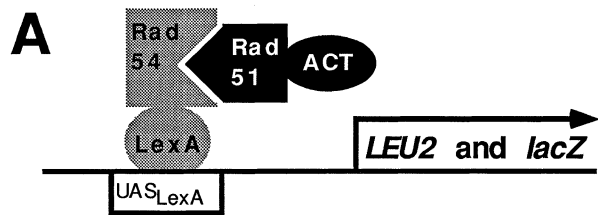
LexA-fusion	Activator-fusion	β-gal. act.
Rad54(1-898)	Rad51(1-400)	124.8 ± 24.1
Rad54(1-356)	Rad51(1-400)	18.3 ± 2.9
Rad54(291-898)	Rad51(1-400)	4.6 ± 2.7
Rad54(1-898)	Rad54(1-898)	3.1 ± 2.5
control	Rad51(1-400)	9.0 ± 2.1

Fig. 1. Interaction between Rad54 and Rad51 proteins in the two-hybrid system. (**A**) The Rad51–activator fusion (Rad51–ACT) is recruited to the promoter by interaction with the LexA–Rad54 fusion to activate transcription of the *LEU2* and *lacZ* reporter genes, both present in the host (Gyuris *et al.*, 1993). (**B**) The activator fusion Rad51(1–400) encoded by pJG4-5-*RAD51*(1–400) contains full-length Rad51p and activates transcription in combination with LexA–Rad54(1–898) encoded by pEG202-*RAD54*(1–898) containing full-length Rad54p. Rad54(1–356) encoded by pEG202-*RAD54*(1–356) is a LexA fusion containing the first 356 amino acids of Rad54p. In Rad54(291–898) encoded by pEG202-*RAD54*(291–898) the C-terminal 608 amino acids of Rad54p were fused to LexA. The activator fusion Rad54(1–898) encoded by pJG4-5-*RAD54*(1–898) contains full-length Rad54p. *Trypanosoma brucei brucei* profilin fused to LexA served as a negative control. β-Galactosidase activity was determined three times in two independent transformants and the mean is expressed in Miller units ± standard deviation.

S.cerevisiae (Figure 1). The Rad51–activator construct activates transcription in combination with LexA–Rad54(1–898) but not with LexA fused to a negative control protein (*Trypanosoma brucei brucei* profilin) or to the C-terminal 607 amino acids of Rad54p [Rad54(291–898)]. Some activation was detected using a LexA fusion to the first 356 amino acids of Rad54p [Rad54(1–356)], with a value significantly different from the others as the standard deviations did not overlap. No evidence for Rad54p–Rad54p interaction was found in this system. Both fusion constructs that showed interaction, pEG202-*RAD54*(1–898) and pJG4-5-*RAD51*(1–400), are biologically active as they complemented the MMS-sensitive phenotype of the respective deletion mutations in *RAD54* and *RAD51*.

Protein complexes containing Rad51 and Rad54 protein exist in vivo

To corroborate this interaction, we demonstrated that complexes containing Rad51 and Rad54 proteins exist in *S.cerevisiae* cells. For this purpose, we used anti-Rad54p antibodies in immunoprecipitation experiments and showed that the two proteins can be co-precipitated (Figure 2A, lanes 4). In a control strain lacking Rad54 protein, no Rad51p was precipitated (Rad51p was tagged with the HA epitope, lanes 5), showing that the HA-Rad51p is not simply precipitating under the experimental
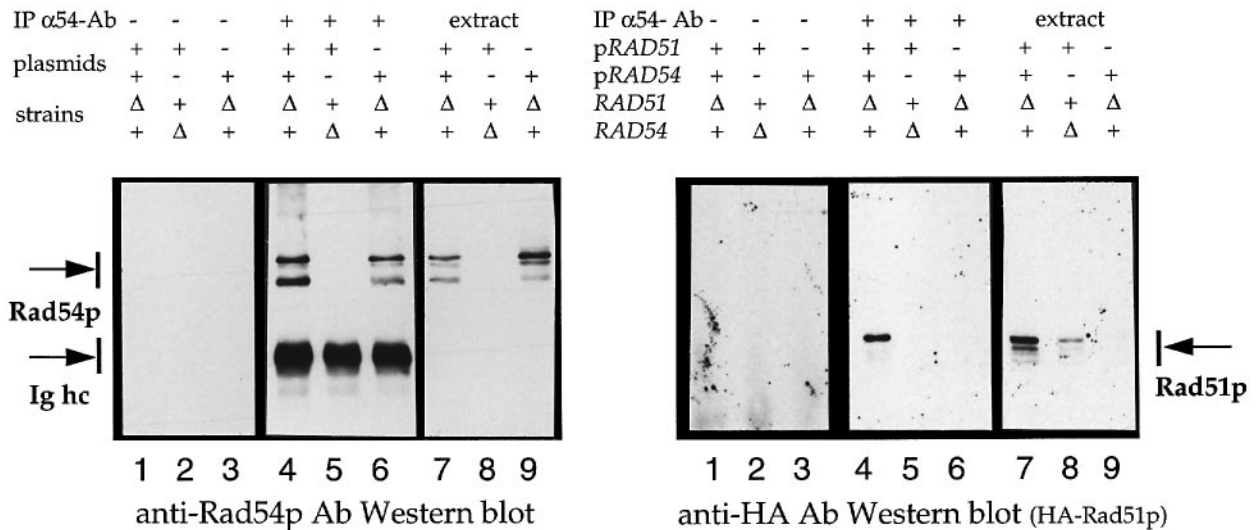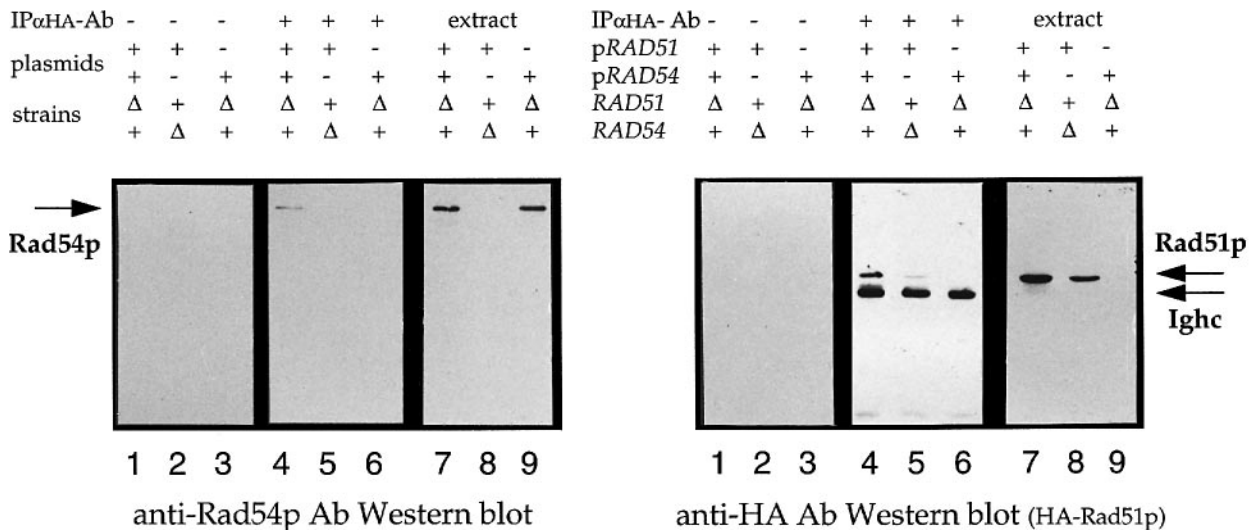
Fig. 2. Rad54 and Rad51 proteins form a complex *in vivo*. (**A**) Immunoprecipitation of lysates from *S.cerevisiae* expressing Rad54p and HA-Rad51p using anti-Rad54 antibodies. Precipitates were split in half, proteins were separated by SDS–PAGE and analyzed by immunoblotting with either anti-Rad54 antibodies (left) or anti-HA antibodies (right). (**B**) Immunoprecipitation of lysates of *S.cerevisiae* expressing Rad54p and HA-Rad51p using anti-HA antibodies. Four-fifths of the precipitate was analyzed using the anti-Rad54 antibodies (left), one-fifth of the precipitate was analyzed using anti-HA antibodies (right) as in (A). Lanes 1–3, immunoprecipitations without first antibody; lanes 4–6, immunoprecipitation containing first antibody; lanes 7–9, direct immunoblot analysis of protein extracts [10 μg in (A), 5 μg in (B)]. Lanes 1, 4 and 7, protein extract from strain FF181079-2 (*rad51Δ*) transformed with pGAL*RAD54* (labeled p*RAD54*) and pJG4-5-*RAD51* (labeled p*RAD51*); lanes 2, 5 and 8, protein extract from strain FF18973 (*rad54Δ*) transformed with pJG4-5-*RAD51* (labeled p*RAD51*); lanes 3, 6 and 9, protein extract from strain FF181079-2 (*rad51Δ*) transformed with pGAL*RAD54* (labeled p*RAD54*). Arrows indicate the positions of the proteins; Ighc denotes immunoglobulin heavy chain.

conditions. In strains lacking HA-Rad51 and Rad51 proteins, Rad54p was precipitated (lane 6 left), but no signal was detected with the anti-HA antibody (lane 6 right). Omitting the anti-Rad54p antibody in the precipitation (lanes 1–3) and analyzing extracts of the strains used (lanes 7–9) by immunoblotting demonstrated the specificity of the antibodies used. The biological significance of the occasional appearance of lower molecular weight forms of Rad54p and HA-Rad51p visible in lanes 4 and 6–9 is unclear. They probably constitute proteolytic degradation products.

Correspondingly, Rad54p–Rad51p-containing complexes were also identified after precipitation of the Rad51 protein partner (Figure 2B, lanes 4). The control experiments demonstrated again the specificity of the antibodies used and the specificity of the interaction. Omitting the anti-HA antibody in the precipitation (lanes 1–3) and analyzing extracts of the strains used (lanes 7–9) demonstrated the specificity of the antibodies in precipitation and immunoblotting. The specificity of the Rad51p–Rad54p association (Figure 2, lanes 4) is demonstrated by the control experiments in lanes 5 and 6,
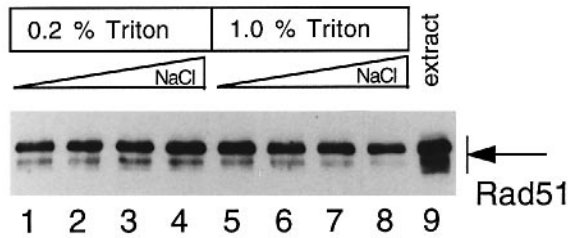
Fig. 3. The Rad51p–Rad54p complex is stable. Immunoprecipitation of lysates from strain FF181079-2 (*rad51Δ*) transformed with pGAL*RAD54* and pJG4-5-*RAD51* expressing Rad54 and HA-Rad51 proteins using anti-Rad54 antibodies. Precipitates were separated by SDS–PAGE and analyzed by immunoblotting with anti-HA antibodies. Lanes 1–4, washes with buffer containing 0.2% Triton X-100; lanes 5–8, washes with buffer containing 1% Triton X-100; lane 9, direct immunoblot analysis of 5 μg of protein extract. Lanes 1 and 5, washes with buffer containing 50 mM NaCl; lanes 2 and 6, 100 mM NaCl; lanes 3 and 7, 150 mM NaCl; and lanes 4 and 8, 200 mM NaCl.

Fig. 4. Rad54p overexpression partially suppresses the UV sensitivity of *rad51Δ*. UV survival curve of strain WDHY1036 (*rad51Δ/rad51Δ*) transformed with pGAL*RAD51* (●), pGal*RAD54* (□), pGal*rad54-K341R* (■) or pGal vector (△) and of strain WDHY669 (wild-type) with pGal vector (○) after plating on medium containing galactose. Shown are averages of experiments from four independent transformants. Standard deviations between WDHY1036 (*rad51Δ*) transformed with pGal*RAD51* and WDHY669 (wild-type) transformed with pGal as well as between WDHY1036 (*rad51Δ*) transformed with pGal and pGal*rad54-K341R* were overlapping, all other standard deviations were not overlapping.

showing that Rad54p is not simply precipitating under the experimental conditions. The amount of HA-Rad51 protein was consistently lower in the absence of Rad54p (compare Figure 2A and B, lanes 7 and 8 right, and in Figure 2B, lanes 4 and 5 right), suggesting a possible stabilization of HA-Rad51p by the complex formation with Rad54p.

In the co-immunoprecipitation experiments, the proteins were overexpressed resulting for Rad54p in a 50- to 100-fold increased protein level compared with the level originating from the chromosomal *RAD54* gene (B.Clever and W.-D.Heyer, unpublished result). The use of the tag precluded the determination of how much HA-Rad51p was overproduced in comparison with native Rad51p. However, co-precipitation of HA-Rad51p with Rad54p did not require such elevated levels of Rad54p, as HA-Rad51p could also be co-precipitated from extracts of a strain containing only the normal chromosomal copy of *RAD54* (data not shown).

To assess the stability of the Rad51p–Rad54p interaction, we performed co-immunoprecipitations using increasingly specific washing conditions. Immunoprecipitations were done exactly as for Figure 2, and precipitates were washed subsequently four times in buffer with increasing concentrations of NaCl and Triton X-100 before immunoblot analysis. As shown in Figure 3, under the most stringent conditions tested (200 mM NaCl/1% Triton X-100), essentially no loss of signal for the co-precipitating HA-Rad51 protein was detected after immunoblot analysis. These conditions are more stringent than *in vivo*. Therefore, the stability of the protein–protein interaction is consistent with the *in vivo* existence of a Rad51p–Rad54p complex.

Co-overexpression of Rad51 and Rad54 proteins causes negative effects in S.cerevisiae

Genetic experiments gave evidence for the biological relevance of the Rad51p–Rad54p interaction. Overexpression of neither Rad54 nor Rad51 proteins alone resulted in negative effects in *S.cerevisiae* (Table I), whereas co-overexpression of both proteins led to a synthetic phenotype of 3-fold reduced survival in the presence of MMS. Mutations in all *RAD52* group genes led to sensitivity against MMS which induces DNA damage repaired by
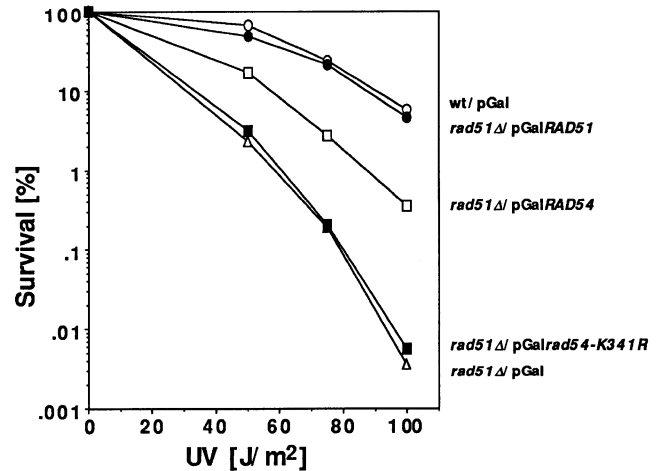
the recombinational repair pathway (reviewed in Friedberg *et al.*, 1995; see also Figure 6).

Furthermore, in the absence of MMS, i.e. under normal growth conditions, co-overexpression of both proteins significantly reduced the cell survival in wild-type diploid cells (see Table I for strains). This can be demonstrated by determining the ratio of the colony-forming units (c.f.u.) on galactose-containing medium (overexpression condition) to the c.f.u. on glucose (non-overexpressing condition). Whereas in double vector control (pGal/YEp13) and single overexpressors (pGal*Rad54*/YEp13, pGal/YEp13*RAD51*) no negative effect on galactose was observed, in cells co-overexpressing both Rad51 and Rad54 proteins survival on galactose was reduced significantly to 0.50 ± 0.15.

Overexpression of Rad54p suppresses some, but not all DNA damage repair defects caused by a deletion in the RAD51 gene

Overexpression of the Swi2p/Snf2p homolog Rad54p in cells lacking the RecA homolog Rad51p surprisingly led

Table I. Negative effect of Rad54p and Rad51p co-overexpression on wild-type *S.cerevisiae*

WDHY669 + plasmids	Survival[a] (%) at:	
	0.005% MMS	0.01% MMS
pGal/YEp13	100 ± 10	103 ± 8
pGal/YEp13*RAD51*	97 ± 8	90 ± 15
pGal*RAD54*/YEp13	100 ± 12	94 ± 16
pGal*RAD54*/YEp13*RAD51*	49 ± 13	32 ± 10

[a]Complete MMS survival curves were performed for six independent transformants with plating on medium containing galactose. The table shows averages ± standard deviations for the relevant data points, the rest of the data are not shown. Survival without MMS was set as 100%.
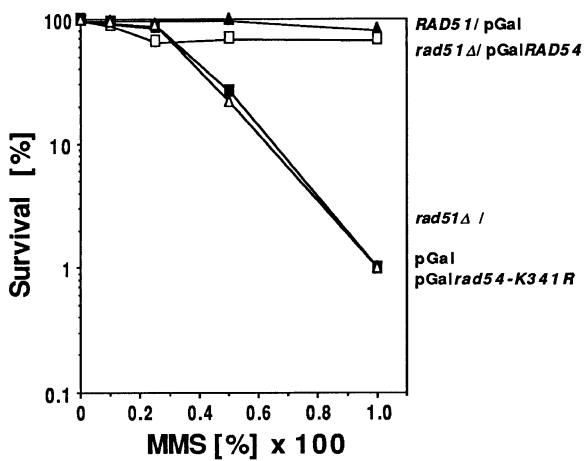
Fig. 5. Genetic interaction between *RAD51* and *RAD54*. Overexpression of Rad54 protein suppresses the MMS sensitivity of *rad51Δ*. Survival of strain FF181079-2 (*rad51Δ*) transformed with either pGal vector (■), pGal*RAD54* (□) or pGal*rad54-K341R* (△) and strain FF18733 (wild-type) transformed with pGal vector (▲) after plating on medium containing glucose and various concentrations of MMS. Shown are the averages of 4–5 independent determinations. At 0.01% MMS, the standard deviations for FF181079 (*rad51Δ*) transformed with pGal vector control (■) and pGal*rad54-K341R* (△) were overlapping as were the standard deviations between FF18733 (wild-type) transformed with pGal vector (▲) and FF181079-2 (*rad51Δ*) transformed with pGal*RAD54*. All other standard deviations were not overlapping. The *rad51Δ* mutation in strain FF181079-2 could be complemented by a plasmid-borne *RAD51* gene.

Fig. 6. Suppression by Rad54p overexpression depends on *RAD55* and *RAD57*. Survival of strains FF18733 (wild-type) transformed with pGal vector, FF181079-2 (*rad51Δ*) transformed with either pGal vector or pGal*RAD54*, WDHY1188 (*rad55Δ rad57Δ*) transformed with either pGal, pGal*RAD54*, or pGal*RAD55-RAD57*, and WDHY960 (*rad51Δ rad55Δ rad57Δ*) transformed with pGal or pGal*RAD54* after plating on plates containing glucose and 0.005% MMS. Complete MMS survival curves (0, 0.001, 0.0025, 0.005 and 0.01% MMS) were performed for 2–5 independent transformants. The figure shows averages ± standard deviations for one data point, the rest of the data are not shown to ease the presentation. Survival without MMS was set as 100%. Absolute values between the experiment shown here and that shown in Figure 5 cannot be compared as different batches of MMS were used for both experiments.

to the suppression of DNA damage repair phenotypes caused by the *rad51Δ* mutation (Figures 4–6). *rad51Δ* causes hypersensitivity against UV irradiation (reviewed by Friedberg *et al.*, 1995; see Figure 4). This phenotype was partially suppressed by Rad54 protein overexpression. At 100 J/m^2, survival of a *rad51Δ* strain is reduced ~1400-fold compared with wild-type. Overexpression of Rad54p in a *rad51Δ* cell restored survival 90-fold. This survival is below that of wild-type cells (16-fold), which is a statistically significant difference.

This suppression effect is dependent on active Rad54 protein, as the *rad54-K341R* mutation abolishes the suppression activity of Rad54p (Figure 4). This mutation affects the central lysine residue in the putative ATP-binding fold (Walker A-box) of the Rad54 protein and abolishes function. The *rad54-K341R* allele did not complement the MMS phenotype of a *rad54Δ* mutant as a centromere-plasmid borne copy or transplaced in the genome (J.Schmuckli-Maurer and W.-D.Heyer, unpublished data).

Moreover, Rad54p overexpression also suppressed the MMS hypersensitivity in *rad51Δ* cells (Figure 5). At 0.01% MMS, survival of the *rad51Δ* strain is reduced 84-fold compared with wild-type. High Rad54p levels improves this 70-fold, to a survival only 1.2-fold below wild-type, which is not statistically significant. As for UV survival, the suppression was dependent on active Rad54p, as the *rad54-K341R* allele did not exhibit this suppression effect (Figure 5).

The defects of *rad51Δ* cells in γ-ray survival, spontaneous and UV-induced mitotic recombination (intragenic recombination *his7-1×his7-2*), and repair of the HO endonuclease-mediated DSB during mating type switching
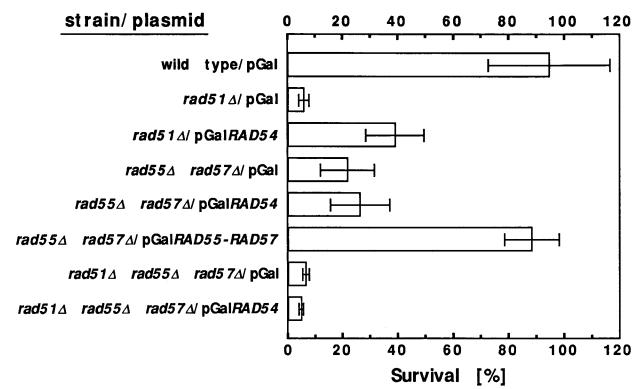
were not suppressed by overexpression of Rad54p (data not shown).

The expression levels of wild-type and mutant Rad54 proteins in cells grown in glucose- and galactose-containing medium were determined by immunoblot analysis (data not shown). The pGal*RAD54* and the pGal*rad54-K341R* vectors resulted in ~10-fold overexpression of proteins in glucose and 50- to 100-fold overexpression in galactose. This analysis therefore established that the loss of suppression activity with *rad54-K341R* was due to non-functional protein, as stable rad54-K341Rp was detectable.

The suppression of *rad51Δ* by high Rad54p levels is genetically dependent on both *RAD55* and *RAD57*

To characterize further the suppression of the *rad51Δ* MMS-hypersensitive phenotype by Rad54p overexpression, we analyzed the genetic requirements for this effect. The suppression was dependent on both the *RAD55* and *RAD57* genes, which encode proteins with sequence similarity to Rad51 and RecA proteins (reviewed in Heyer, 1994).

Mutations in *RAD55* and/or *RAD57* exhibit overlapping phenotypes which are less severe than those of *rad51*, *rad52* or *rad54* mutants (compare in Figure 6 *rad51Δ* with *rad55Δ rad57Δ*). This is especially the case at 30°C, the temperature used here, as deletions in *RAD55* or *RAD57* exhibit an unusual, cold-sensitive phenotype (Lovett and Mortimer, 1987). The triple mutant (*rad51Δ rad55Δ rad57Δ*) exhibits exactly the same MMS sensitivity as the *rad51Δ* single mutant (see Figure 6). Overexpression of Rad54p in *rad51Δ* cells again showed the suppression (see Figure 6 and legend) already seen in Figure 5. Overexpression of Rad54p in the triple mutant (*rad51Δ rad55Δ rad57Δ*; Figure 6) or in either double mutant (*rad51Δ rad57Δ*, *rad51Δ rad55Δ*; data not shown) did not have

any effect on the MMS sensitivity of the cells. Based on this quantitative difference, we conclude that the Rad54p suppression effect depends on *RAD55* and *RAD57*. If this effect were independent of Rad55 and Rad57 proteins, the survival in the triple mutant strain overexpressing Rad54p should have been equal to the survival of the *rad55*Δ *rad57*Δ double mutant strain, which is clearly not the case (see Figure 6). To exclude a possible negative effect of Rad54p overexpression in a *rad55*Δ *rad57*Δ background, we tested this possibility and no negative effect was detected (see Figure 6).

The *rad55*Δ *rad57*Δ mutations could be complemented to wild-type level by a plasmid expressing both proteins (Figure 6). Overexpression of Rad54p did not suppress the MMS sensitivity of *rad55*, *rad57 or rad55 rad57* mutants under the conditions analyzed (25 or 30°C, glucose or galactose; data not shown).

Discussion

Rad51 and Rad54 proteins form a complex in vivo

Recombinational repair of DSBs and other lesions to DNA in *S.cerevisiae* is controlled by the evolutionarily conserved *RAD52* group of genes. Rad51 and Rad54 proteins perform crucial functions in this process (reviewed by Game, 1993). Here we provide strong evidence that Rad51 and Rad54 proteins interact *in vivo* (Figures 1–3). The interaction measured in the two-hybrid system is specific and requires a distinct region of Rad54p. Rad54 protein is organized grossly in two regions, an N-terminal region (amino acids 1–326) called Rad54A and a C-terminal region (amino acids 327–898) called Rad54B. Rad54B includes the region of sequence homology to the Swi2p/Snf2p family and the putative DNA helicase motifs (Eisen *et al.*, 1995), whereas Rad54A is specific for Rad54p and shows only significant sequence homology to Rad54 proteins from other organisms (Kanaar *et al.*, 1996; Muris *et al.*, 1996). The data from the two-hybrid system suggest that the Rad54A part may be involved in the Rad51p interaction, whereas no indication for an involvement of the Rad54B part has been obtained in this system. The B-part fusion was found to be as abundant as the full-length fusion protein in the cell (data not shown). Unrelated two-hybrid experiments also suggested that the Rad54B fusion is stable *in vivo* (H.Interthal and W.-D.Heyer, unpublished results). As no other protein in *S.cerevisiae* shares significant homology with the Rad54A part, it is unlikely that the interaction with Rad51p is fortuitous. This might have been argued had the interaction involved the Rad54B part, as several *S.cerevisiae* proteins share significant homology with Rad54p in this region. Moreover, using a Rad54p fragment encompassing the first 114 amino acids, *in vitro* interaction with purified Rad51p was observed (A.Shinohara, personal communication). Therefore, the Rad51p-specific interaction appears to be mediated by the N-terminal region of Rad54p.

Functional interaction between Rad51 and Rad54 proteins

The genetic experiments reported here (Figures 4–6; Table I) provide compelling evidence that the Rad51p–Rad54p interaction is functionally significant for the mechanism of recombinational repair. Rad51p, like its prokaryotic counterpart RecA protein, is probably active in the central step of recombinational repair, i.e. search for homology and homologous pairing. Rad51p is a DNA-dependent ATPase that forms nucleoprotein filaments active in homology search *in vitro* (Ogawa *et al.*, 1993; Sung, 1994; Sung and Robberson, 1995). Rad54p has no prokaryotic counterpart to serve as a paradigm. However, information is available about two other *S.cerevisiae* members of the Swi2p/Snf2p family. Swi2p/Snf2p itself is a DNA-stimulated ATPase that acts in a high molecular weight complex involved in overcoming the general repressive effect of chromatin on transcription (reviewed in Peterson and Tamkun, 1995). Mot1p, another family member, is also a DNA-stimulated ATPase that is capable of removing TATA-binding protein (TBP) from DNA, thereby resulting in global repression of RNA polymerase II transcription (Auble *et al.*, 1994). Despite some superficial similarities between Rad51p, Swi2p/Snf2p and Mot1p being DNA-dependent/stimulated ATPases, Rad51p is different from the other proteins (see Kowalczykowski and Eggleston, 1994; Shinohara and Ogawa, 1995). Therefore, it was surprising to find that high Rad54p levels suppress certain DNA damage repair defects of a deletion in the *RAD51* gene. Suppression was found for the MMS and UV sensitivity but not for the γ-ray sensitivity, the spontaneous and UV-induced mitotic recombination defects, and the mating type switching defect caused by a deletion of the *RAD51* gene. These results point to a heterogeneity in the genetic requirements within the *RAD52* pathway for different types of DNA damage repair and different genetic endpoints.

The Rad54p-mediated suppression of certain DNA damage repair-related phenotypes caused by *rad51*Δ could either be direct, by partial functional replacement of Rad51p by Rad54p, or indirect, involving other proteins. Recently, the crystal structure of the PcrA protein from *Bacillus stearothermophilus* has been solved (Subramanya *et al.*, 1996). PcrA protein is a DNA helicase with seven sequence domains shared by many DNA helicases and other DNA-dependent or stimulated ATPases including Rad54p (see Gorbalenya and Koonin, 1993). Surprisingly, the ATP-binding domain of this DNA helicase was structurally highly similar to that of the *Escherichia coli* RecA protein (Story and Steitz, 1992; Story *et al.*, 1992), as pointed out by Subramanya *et al.* (1996). As Rad51 protein forms nucleoprotein filaments equivalent to RecA–DNA filaments (Ogawa *et al.*, 1993) and has an amino acid sequence that can be modeled into the RecA crystal structure (Story *et al.*, 1993), partial functional replacement of Rad51p by Rad54p cannot be dismissed. Such an interpretation would predict that this effect is autonomous, i.e. independent of other genes. Testing this hypothesis, we found that the suppression by high Rad54p levels was dependent on Rad55 and Rad57 proteins. Therefore, the suppression is not achieved by functional replacement but rather by an indirect route involving other proteins. The inability of Rad51p overexpression to suppress a deletion in the *RAD54* gene (data not shown) is consistent with this interpretation. Interestingly, *RAD54* has been isolated as a high copy suppressor of the meiotic arrest and spore inviability phenotypes of a *dmc1* mutant (D.Bishop, personal communication). Dmc1p is another *S.cerevisiae*

protein with homology to *E.coli* RecA that specifically functions during meiosis (Bishop *et al.*, 1992).

The role of Rad55 and Rad57 proteins

Functional suppression of a *rad51Δ* mutation by high levels of Rad54p involves both Rad55 and Rad57 proteins. They are also members of the recombinational repair group (reviewed in Friedberg *et al.*, 1995; Shinohara and Ogawa, 1995) and exhibit overlapping mutant phenotypes which are less severe than those of *rad51*, *rad52* or *rad54* mutants. Rad55p (Lovett, 1994) and Rad57p (Kaus and Mortimer, 1991) share certain sequence similarity with *E.coli* RecA and Rad51p (reviewed in Heyer, 1994; Shinohara and Ogawa, 1995). Both proteins have been shown to interact (Hays *et al.*, 1995; Johnson and Symington, 1995), consistent with our result of their mutual requirement for the Rad54p-mediated suppression effect. In addition, Rad55p and Rad51p interact (Hays *et al.*, 1995; Johnson and Symington, 1995). Overexpression of Rad51p can partially suppress DNA damage repair phenotypes of mutations in the *RAD55* and *RAD57* genes (Hays *et al.*, 1995; Johnson and Symington, 1995), but Rad54p overexpression does not. Our data are the first evidence that Rad55 and Rad57 proteins correspondingly are involved in suppression of a *rad51* mutation, at least under conditions of high Rad54p levels. The molecular details of these complex interactions between four recombinational repair proteins remain to be determind. Rad55 and Rad57 proteins may serve as accessory factors to the Rad51p–DNA filament during homology search, providing functions similar to, and partially redundant with, Rad51p during recombinational repair. Alternatively, these data might be interpreted to mean that deletion of *RAD55* and/or *RAD57* affects MMS sensitivity independently of *rad51*. However, such an interpretation is not consistent with the fact that the *rad51* mutation is epistatic to the *rad55* and/or *rad57* mutation, as the single *rad51* mutation has the same MMS sensitivity as the *rad51 rad55 rad57* triple mutant (Figure 6) or the *rad51 rad55* and *rad51 rad57* double mutants (data not shown).

Protein–protein interactions in recombinational repair: possible roles for Rad54p

Specific protein–protein interactions are important in numerous DNA metabolic processes, including DNA replication (reviewed in Kornberg and Baker, 1992), mismatch repair (reviewed in Kolodner, 1996), transcription (see Struhl, 1995), NER (reviewed in Wood, 1996) and recombination in *E.coli* (reviewed in Kowalczykowski *et al.*, 1994) and phage T4 (reviewed in Kowalczykowski and Eggleston, 1994). Therefore, it is not surprising to find that protein–protein interactions play a significant role during recombinational repair in eukaryotes. Identifying the protein partners interacting in a putative recombinational repair complex or complexes and understanding their mutual interactions will give important insights into the mechanism of this process. Multiple individual interactions between recombinational repair proteins (Rad51p–Rad52p, Shinohara *et al.*, 1992; Rad51p–Rad55p, Rad55p–Rad57p, Hays *et al.*, 1995; Johnson and Symington, 1995; Rad52p–Rpa1p, Firmenich *et al.*, 1995; for additional references see above; Rad51p–Rad54p, this study) have been identified in *S.cerevisiae*. They provide

a functional framework which has been interpreted as evidence for a 'putative recombinosome' including heterotrimeric Rpa, Rad51, Rad52, Rad54, Rad55 and Rad57 proteins (Hays *et al.*, 1995; this study). It is unclear presently whether these proteins are assembled in one complex or in different sub-complexes. The existence of different sub-complexes may be indicated by the heterogeneity in the genetic requirement found in the suppression of *rad51* phenotypes by Rad54p overexpression. In addition, these individual interactions might not necessarily all occur simultaneously. The existence of a large 'repairosome' in NER (Svejstrup *et al.*, 1995) recently has been questioned (Guzder *et al.*, 1996). The individual interactions discussed above, and in particular the interaction between Rad51 and Rad54 proteins, might be relevant at several steps during recombinational repair. The biochemical data and the RecA paradigm strongly suggest a role for the Rad51p–DNA filament in homology search and pairing, a central step in this process. Rad54p might be involved in the assembly of such a filament as an assembly factor or molecular matchmaker (see Sancar and Hearst, 1993). Alternatively or in addition, Rad54p may have an active role during Rad51p-mediated homology search as an accessory protein similar to gp41 helicase in phage T4 recombination (Salinas and Kodadek, 1995). It has been suggested that Rad54p (and Rad51, Rad55, Rad57 proteins) are accessory proteins needed to gain access to otherwise inaccessible regions of the chromosome (Sugawara *et al.*, 1995), possibly in a way similar to the role of Swi2p/Snf2p in overcoming transcriptional repression by chromatin (reviewed by Peterson and Tamkun, 1995). Finally, Rad54p could act in the turnover of the Rad51 protein–DNA filament in analogy to the action of Mot1p during RNA polymerase II transcription (see Auble *et al.*, 1994). This would allow assembly of the crucial Rad51p filament at the site of DNA damage, which under conditions of limiting Rad51p concentration might result in severe mutant phenotypes for *rad54*. A similar speculation might explain the suppression of *rad51Δ* by high levels of Rad54p through an increased turnover of Rad55 and Rad57 proteins.

Materials and methods

Strains, media and growth conditions

The *S.cerevisiae* strains used in this study are listed in Table II. Standard media and standard growth conditions were as described (Sherman *et al.*, 1982; Bähler *et al.*, 1994). MMS was added to solid medium cooled to 50°C before pouring, and plates were used within 1 day. The *S.cerevisiae* strains were transformed using the lithium acetate method modified according to Schiestl and Gietz (1989).

Plasmid constructions and DNA methods

Plasmids were constructed by standard methods. For the LexA fusions: pEG202-*RAD54*(1–898) contains the *Nco*I (Klenow resected)–*Sal*I *RAD54* fragment of pWDH251 (Kanaar *et al.*, 1996) in *Eco*RI (Klenow fill in)–*Sal*I-digested pEG202 (Gyuris *et al.*, 1993); pEG202-*RAD54*(1–356) derives from pEG202-*RAD54*(1–898) by *Apa*I–*Sal*I digestion, Klenow fill-in and religation; pEG202-*RAD54*(291–898) is the *Rca*I (Klenow fill-in)–*Sal*I *RAD54* fragment of pEG202-*RAD54*(1–898) in *Eco*RI (Klenow fill-in)–*Sal*I-digested pEG202.

Activator fusions: pJG4-5-*RAD51*(1–400) contains an *Eco*RI (partial cleavage)–*Sal*I *RAD51* fragment of pEG202-*RAD51* in *Eco*RI–*Xho*I-digested pJG4-5 (Gyuris *et al.*, 1993); pEG202-*RAD51* was constructed by inserting the *Stu*I–*Dra*I *RAD51* fragment of YEp13-RAD51-23 (Calderon *et al.*, 1983) in the *Sma*I site of pEG202; pJG4-5-*RAD54*(1–

Table II. *Saccharomyces cerevisiae* strains

Strain	Genotype
EGY48[a]	**a** *trp1 ura3-52 his3 LEU2::pLexAop6-LEU2*
FF18973[b]	**a** *leu2-3,-112 trp1-289 ura3-52 his7-2 lys1-1*
FF18733[b]	**a** *leu2-3,-112 trp1-289 ura3-52 his7-2 lys1-1 rad54Δ::LEU2*
FF181079-2[b]	**a** *leu2-3,-112 trp1-289 ura3-52 his7-2 lys1-1 rad51Δ::ura3*
WDHY669	**a/α** *leu2-3,-112/leu2-3,-112 trp1-289/+ ura3-52/ura3-52 his7-1/his7-2 lys1-1/+ +/lys2-1*
WDHY911	**a** *leu2-3,-112 trp1-289 ura3-52 his7-2 lys1-1 rad51Δ::ura3 rad55Δ::kanMX*
WDHY913	**a** *leu2-3,-112 trp1-289 ura3-52 his7-2 lys1-1 rad51Δ::ura3 rad57Δ::kanMX*
WDHY960	**a** *leu2-3,-112 trp1-289 ura3-52 his7-2 lys1-1 rad51Δ::ura3 rad55Δ::kanMX rad57Δ::kanMX*
WDHY1036	**a/α** *leu2-3,-112/leu2-3,-112 trp1-289/trp1-289 ura3-52/ura3-52 his7-1/his7-2 lys1-1/+ +/lys2-1 rad51Δ::ura3/rad51Δ::kanMX*
WDHY1188	**a** *leu2-3,-112 trp1-289 ura3-52 his7-2 lys1-1 rad55Δ::kanMX rad57Δ::kanMX*

[a]Gyuris *et al.* (1993).
[b]From F.Fabre, Institut Curie, Paris.
Strains where no reference is given were constructed for this study. All WDHY strains used here are isogenic derivates obtained by transformation of the FF strains kindly supplied by Dr Fabre.

898) contains the *RAD54 Eco*RI fragment of pEG202-*RAD54* in *Eco*RI-digested pJG4-5.

pGal vector is pWDH181 described previously (Bashkirov *et al.*, 1995), and YEp13 is described in Broach *et al.* (1979). pGal*RAD51* was constructed by ligating the *Stu*I–*Dra*I 1.4 kb *RAD51* fragment of YEp13-*RAD51*-23 (Calderon *et al.*, 1983) into pWDH129 (Holler *et al.*, 1995) cut with *Eco*RI and *Nhe*I after fill-in with Klenow. pWDH251 expresses a His(6)-tagged Rad54 protein using the pT7-7 system (Tabor and Richardson, 1985). The *Xho*I–*Eco*RI *RAD54* fragment of pGal*RAD54* was cloned in the *Xho*I–*Eco*RI-digested pTrcHisC (Invitrogen) to result in pWDH202. The *Nco*I (partial, Klenow fill-in)–*Eco*RI fragment encoding His(6)-Rad54 of pWDH202 was cloned in *Nde*I (Klenow fill-in)–*Eco*RI-digested pT7-7 to result in pWDH251. pGal*RAD55*-*RAD57* also derives from pWDH181, placing *RAD55* and *RAD57* individually under the control of the Gal promoter in a head-to-head fashion (J.King and W.-D.Heyer, unpublished; details to be published elsewhere).

The *rad54-K341R* allele was generated by site-directed mutagenesis using the oligonucleotide 5′-G CTG GGT AGG ACA TTG C-3′, otherwise pGal*rad54-K341R* is identical to pGal*RAD54*. The *rad51Δ::kanMX*, *rad55Δ::kanMX* and *rad57Δ::kanMX* alleles were generated by PCR product-mediated transformation as described (Wach *et al.*, 1994), deleting the entire open reading frame.

Genetic and physiological methods

The two-hybrid system in *S.cerevisiae* strain EGY48 was used as described previously (Gyuris *et al.*, 1993). Quantitative β-galactosidase assays were performed as described (Harshman *et al.*, 1988). The LexA activator fusions did not appreciably induce reporter gene expression by themselves. They exhibited activity in the repression assay, demonstrating that the respective LexA fusion proteins were synthesized, transported to the nucleus and active in binding the LexA operator.

To establish MMS survival curves, transformants were grown in minimal medium selective for the presence of the plasmids to stationary phase, diluted in H$_2$O, and aliquots were plated on medium lacking leucine and/or uracil with glucose or galactose (2% w/v) as carbon source and the indicated concentration of MMS. UV survival curves were performed at fluencies of 0–100 J/m^2 on plates containing galactose. Colonies were counted after 6 days of incubation at 30°C. γ-Ray survival curves were performed at 0–200 Gray irradiating the cells in solution using a ^{137}Cs source and plating appropriate dilutions on plates containing glucose and galactose.

Recombination rates between the *his7-1* and *his7-2* hetero-alleles were determined by fluctuation tests using the method of the median (Lea and Coulson, 1949). For measurement of UV-induced recombination, cells were irradiated with 25 J/m^2. Repair of the HO endonuclease-mediated DSB at the *MAT* locus was monitored as survival after inducing the *HO* gene controlled by the *GAL1-10* promoter and determining the number of colonies on medium containing glucose and galactose.

Protein methods

For co-immunoprecipitation experiments, strain FF181079-2 transformed with plasmid pGal*RAD54* (labeled p*RAD54* in Figure 2) and pJG4-5-*RAD51* (labeled p*RAD51* in Figure 2) as well as strain FF18973 transformed with either plasmid were grown in medium selecting for the presence of the plasmids containing raffinose (2% w/v). Both

plasmids encoded functional Rad51 and Rad54 proteins as they complemented deletion mutations in the respective genes. Five hours after addition of galactose (2% w/v), cells were harvested, washed, resuspended in lysis buffer (20 mM HEPES pH 7.4, 100 mM KOAc, 2 mM MgOAc) and frozen in liquid N$_2$. Fifty OD units of frozen cells were thawed on ice, resuspended in 200 µl of lysis buffer containing 2 mM phenylmethylsulfonyl fluoride (PMSF), and extracted using 0.45 µm glass beads. Three hundred µg of extract were mixed with 500 µl of IP buffer (50 mM Tris–HCl pH 7.5, 0.2 % Triton X-100, 50 mM NaCl) containing 0.5 mg/ml bovine serum albumin (BSA), to which either 0 or 1 µg of anti-Rad54 antibodies or 0 or 5 µg of anti-HA antibodies (12CA5, Boehringer Mannheim) were added. After overnight rocking at 4°C, 100 µl of protein G–Sepharose FF (Pharmacia) slurry previously washed in IP buffer were added and incubated for 1 h. Immunoprecipitates were washed twice with 500 µl of IP buffer containing BSA and twice in IP buffer before the beads were resuspended in 1× Laemmli buffer. Proteins were separated by SDS–PAGE. Immunoblot detection was by ECL (Amersham). Additional co-immunoprecipitation experiments using cells not overexpressing Rad54p were done exactly as above but using 4 mg of whole cell extract. Purification of the recombinant His(6)-Rad54 protein from *E.coli* cells transformed with pWDH251, production of polyclonal antisera in rabbits and immunoaffinity purification of antibodies were done essentially as described (Santos-Rosa *et al.*, 1996) and will be described fully elsewhere (B.Clever, J.Schmuckli-Maurer and W.-D.Heyer, in preparation).

References

Aboussekhra,A.R., Chanet,R., Adjiri,A. and Fabre,F. (1992) Semi-dominant suppressors of *srs2* helicase mutations of *Saccharomyces cerevisiae* map in the *RAD51* gene, whose sequence predicts a protein with similarities to procaryotic RecA proteins. *Mol. Cell. Biol.*, **12**, 3224–3234.

Auble,D.T., Hansen,K.E., Mueller,C.G.F., Lane,W.S., Thorner,J. and Hahn,S. (1994) Mot1, a global repressor of RNA polymerase II transcription, inhibits TBP binding to DNA by an ATP-dependent mechanism. *Genes Dev.*, **8**, 1920–1934.

Bähler,J., Hagens,G., Holzinger,G., Scherthan,H. and Heyer,W.-D. (1994) *Saccharomyces cerevisiae* cells lacking the homologous pairing protein p175^{SEP1} arrest at pachytene during meiotic prophase. *Chromosoma*, **103**, 129–141.

Bashkirov,V.I., Solinger,J.A. and Heyer,W.-D. (1995) Identification of functional domains in the Sep1 protein (= Kem1, Xrn1), which is required for transition through meiotic prophase in *Saccharomyces cerevisiae*. *Chromosoma*, **104**, 215–222.

Basile,G., Aker,M. and Mortimer,R.K. (1992) Nucleotide sequence and transcriptional regulation of the yeast recombinational repair gene *RAD51*. *Mol. Cell. Biol.*, **12**, 3235–3246.

Bendixen,C., Sunjeyaric,I., Bauchwitz,R. and Rothstein,R. (1994) Identification of a mouse homologue of the *Saccharomyces cerevisiae* recombination and repair gene, *RAD52*. *Genomics*, **23**, 300–303.

Bezzubova,O.Y., Schmidt,H., Ostermann,K., Heyer,W.-D. and Buerstedde,J.-M. (1993) Identification of a chicken *RAD52* homologue suggests conservation of the *RAD52* recombination pathway throughout the evolution of higher eukaryotes. *Nucleic Acids Res.*, **21**, 5945–5949.

Bishop,D.K., Park,D., Xu,L. and Kleckner,N. (1992) *DMC1*, a meiosis-specific yeast homolog of *E.coli* recA required for recombination, synaptonemal complex formation, and cell cycle progression. *Cell*, **69**, 439–456.

Broach,J.R., Strathern,J.N. and Hicks,J.B. (1979) Transformation in yeast: development of a hybrid cloning vector and isolation of the *CAN1* gene. *Gene*, **8**, 121–133.

Calderon,I.L., Contopoulou,C.R. and Mortimer,R.K. (1983) Isolation and characterization of yeast repair genes. II. Isolation of plasmids that complement the mutations *rad50-1*, *rad51-1*, *rad54-3*, and *rad55-3*. *Curr. Genet.*, **7**, 93–100.

Donovan,J.W., Milne,G.T. and Weaver,D.T. (1994) Homotypic and heterotypic protein associations control Rad51 function in double-strand break repair. *Genes Dev.*, **8**, 2552–2562.

Eisen,J.A., Sweder,K.S. and Hanawalt,P.C. (1995) Evolution of the SNF2 family of proteins: subfamilies with distinct sequences and functions. *Nucleic Acids Res.*, **23**, 2715–2723.

Emery,H.S., Schild,D., Kellogg,E. and Mortimer,R.K. (1991) Sequence of *RAD54*, a *Saccharomyces cerevisiae* gene involved in recombination and repair. *Gene*, **104**, 103–106.

Fields,S. and Song,O.-K. (1989) A novel genetic system to detect protein–protein interactions. *Nature*, **340**, 245–246.

Firmenich,A.A., Elias-Arnanz,M. and Berg,P. (1995) A novel allele of *Saccharomyces cerevisiae RFA1* that is deficient in recombination and repair and suppressible by *RAD52*. *Mol. Cell. Biol.*, **15**, 1620–1631.

Fishman-Lobell,J., Rudin,N. and Haber,J.E. (1992) Two alternative pathways of double-strand break repair that are kinetically separable and independently modulated. *Mol. Cell. Biol.*, **12**, 1292–1303.

Friedberg,E.C., Walker,G.C. and Siede,W. (1995) *DNA Repair and Mutagenesis*. American Society for Microbiology, Washington, DC.

Game,J.C. (1983) Radiation-sensitive mutants and repair in yeast. In Spencer,J.F.T., Spencer,D. and Smith,A.R.W. (eds), *Yeast Genetics, Fundamental and Applied Aspects*. Springer-Verlag, New York, pp. 109–137.

Game,J.C. (1993) DNA double-strand breaks and the *RAD50–RAD57* genes in *Saccharomyces*. *Semin. Cancer Biol.*, **4**, 73–83.

Gorbalenya,A.E. and Koonin,E.V. (1993) Helicases: amino acid sequence comparisons and structure–function relationships. *Curr. Opin. Struct. Biol.*, **3**, 419–429.

Guzder,S.N., Sung,P., Prakash,L. and Prakash,S. (1996) Nucleotide excision repair in yeast is mediated by sequential assembly of repair factors and not by a pre-assembled repairosome. *J. Biol. Chem.*, **271**, 8903–8910.

Gyuris,J., Golemis,E.A., Chertkov,H. and Brent,R. (1993) Cdi1, a human G1 and S phase protein phosphatase that associates with Cdk2. *Cell*, **75**, 791–803.

Haber,J.E. (1992) Exploring the pathways of homologous recombination. *Curr. Opin. Cell. Biol.*, **4**, 401–412.

Harshman,K.D., Moye-Rowley,W.S. and Parker,C.S. (1988) Transcriptional activation by the SV40 AP-1 recognition element in yeast is mediated by a factor similar to AP-1 that is distinct from GCN4. *Cell*, **53**, 321–330.

Haynes,R.H. and Kunz,B.A. (1981) DNA repair and mutagenesis in yeast. In Strathern,J.N., Jones,E.W. and Broach,J.R. (eds), *The Molecular Biology of the Yeast Saccharomyces*, *Life Cycle and Inheritance*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 371–414.

Hays,S.L., Firmenich,A.A. and Berg,P. (1995) Complex formation in yeast double-strand break repair: participation of Rad51, Rad52, Rad55, and Rad57 proteins. *Proc. Natl Acad. Sci. USA*, **92**, 6925–6929.

Heyer,W.-D. (1994) The search for the right partner: homologous pairing and DNA strand exchange proteins in eukaryotes. *Experientia*, **50**, 223–233.

Hoeijmakers,J.H.J. (1993) Nucleotide excision repair II: from yeast to mammals. *Trends Genet.*, **9**, 211–217.

Holler,A., Bashkirov,V.I., Solinger,J.A., Reinhart,U. and Heyer,W.-D. (1995) Use of monoclonal antibodies in the functional characterization of the *Saccharomyces cerevisiae* Sep1 protein. *Eur. J. Biochem.*, **231**, 329–336.

Johnson,R.D. and Symington,L.S. (1995) Functional differences and interactions among the putative RecA homologs Rad51, Rad55, and Rad57. *Mol. Cell. Biol.*, **15**, 4843–4850.

Kanaar,R. *et al.* (1996) Human and mouse homologs of the *Saccharomyces cerevisiae RAD54* DNA repair gene: evidence for functional conservation. *Curr. Biol.*, **6**, 828–838.

Kaus,J.A. and Mortimer,R.K. (1991) Nucleotide sequence of the *RAD57* gene of *Saccharomyces cerevisiae*. *Gene*, **105**, 139–140.

Kleckner,N., Padmore,R. and Bishop,D.K. (1991) Meiotic chromosome metabolism: one view. *Cold Spring Harbor Symp. Quant. Biol.*, **56**, 729–743.

Kolodner,R. (1996) Biochemistry and genetics of eukaryotic mismatch repair. *Genes Dev.*, **10**, 1433–1442.

Kornberg,A. and Baker,T.A. (1992) *DNA Replication*. W.H.Freeman and Company, NY.

Kowalczykowski,S.C. and Eggleston,A.K. (1994) Homologous pairing and DNA strand exchange proteins. *Annu. Rev. Biochem.*, **63**, 991–1043.

Kowalczykowski,S.C., Dixon,D.A., Eggleston,A.K., Lauder,S.D. and Rehrauer,W.M. (1994) Biochemistry of homologous recombination in *Escherichia coli*. *Microbiol. Rev.*, **58**, 401–465.

Kramer,K.M., Brock,J.A., Bloom,K., Moore,J.K. and Haber,J.E. (1994) Two different types of double-strand breaks in *Saccharomyces cerevisiae* are repaired by similar *RAD52*-independent, non-homologous recombination events. *Mol. Cell. Biol.*, **14**, 1293–1301.

Lea,D.E. and Coulson,C.A. (1949) The distribution of the numbers of mutants in bacterial populations. *J. Genet.*, **49**, 264–285.

Lovett,S.T. (1994) Sequence of the *RAD55* gene of *Saccharomyces cerevisiae*: similarity of RAD55 to prokaryotic RecA and other RecA-like proteins. *Gene*, **142**, 103–106.

Lovett,S. and Mortimer,R.K. (1987) Characterization of null mutants of the *RAD55* gene of *Saccharomyces cerevisiae*: effects of temperature, osmotic strength, and mating type. *Genetics*, **116**, 547–553.

Milne,G.T. and Weaver,D.T. (1993) Dominant-negative alleles of *RAD52* reveal a DNA repair/recombination complex including Rad51 and Rad52. *Genes Dev.*, **7**, 1755–1765.

Muris,D.F.R. *et al.* (1994) Cloning of human and mouse genes homologous to *RAD52*, a yeast gene involved in DNA repair and recombination. *Mutat. Res.*, **315**, 295–305.

Muris,D.F.R., Vreeken,K., Carr,A.M., Murray,J.M., Smit,C., Lohman,P.H.M. and Pastink,A. (1996) Isolation of the *Schizosaccharomyces pombe RAD54* homologue, *rhp54*$^+$, a gene involved in the repair of radiation damage and replication fidelity. *Cell Sci.*, **109**, 73–81.

Ogawa,T., Yu,X., Shinohara,A. and Egelman,E.H. (1993) Similarity of the yeast RAD51 filament to the bacterial RecA filament. *Science*, **259**, 1896–1899.

Peterson,C.L. and Tamkun,J.W. (1995) The SWI–SNF complex: a chromatin remodeling machine? *Trends Biochem. Sci.*, **20**, 143–146.

Petes,T.D., Malone,R.E. and Symington,L.S. (1991) Recombination in yeast. In Broach,J.R., Pringle,J.R. and Jones,E.W. (eds), *The Molecular and Cellular Biology of the Yeast Saccharomyces: Genome Dynamcis, Protein Synthesis*, *and Energetics*. Cold Spring Habor Laboratory Press, Cold Spring Harbor, NY, pp. 407–521.

Resnick,M.A. (1976) The repair of double-strand breaks in DNA: a model involving recombination. *J. Theor. Biol.*, **59**, 97–106.

Roeder,G.S. (1995) Sex and the single cell: meiosis in yeast. *Proc. Natl Acad. Sci. USA*, **92**, 10450–10456.

Roth,D. and Wilson,J. (1988) Illegitimate recombination in mammalian cells. In Kucherlapati,R. and Smith,G.R. (eds), *Genetic Recombination*. American Society for Microbiology, Washington, DC, pp. 621–653.

Salinas,F. and Kodadek,T. (1995) Phage T4 homologous strand exchange: a DNA helicase, not the strand transferase, drives polar branch migration. *Cell*, **82**, 111–119.

Sancar,A. and Hearst,J.E. (1993) Molecular matchmakers. *Science*, **259**, 1415–1420.

Sandler,S.J., Satin,L.H., Samra,H.S. and Clark,A.J. (1996) *recA*-like genes from three archaean species with putative protein products similar to Rad51 and Dmc1 proteins of the yeast *Saccharomyces cerevisiae. Nucleic Acids Res.*, **24**, 2125–2132.

Santos-Rosa,H., Clever,B., Heyer,W.-D. and Aguilera,A. (1996) The yeast *HRS1* gene encodes a polyglutamine-rich nuclear protein required for spontaneous and *hpr1*-induced deletion between direct repeats. *Genetics*, **142**, 705–716.

Schiestl,R.S. and Gietz,R.D. (1989) High efficiency transformation of intact yeast cells using single stranded nucleic acids as a carrier. *Curr. Genet.*, **16**, 339–346.

Schild,D. (1995) Suppression of a new allele of the yeast *RAD52* gene by overexpression of *RAD51*, mutations in *srs2* and *ccr4*, or mating-type heterozygosity. *Genetics*, **140**, 115–127.

Sherman,F., Fink,G.R. and Hicks,J.B. (1982) *Methods in Yeast Genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Shinohara,A., Ogawa,H. and Ogawa,T. (1992) Rad51 protein involved in repair and recombination in *S.cerevisiae* is a RecA-like protein. *Cell*, **69**, 457–470.

Shinohara,A., Ogawa,H., Matsuda,Y., Ushio,N., Ikeo,K. and Ogawa,T. (1993) Cloning of human, mouse and fission yeast recombination genes homologous to RAD51 and recA. *Nature Genet.*, **4**, 239–243.

Shinohara,A. and Ogawa,T. (1995) Homologous recombination and the roles of double-strand breaks. *Trends Biochem. Sci.*, **20**, 387–391.

Story,R.M. and Steitz,T.A. (1992) Structure of the recA protein–ADP complex. *Nature*, **355**, 374–376.

Story,R.M., Weber,I.T. and Steitz,T.A. (1992) The structure of the *E.coli* recA protein monomer and polymer. *Nature*, **355**, 318–325.

Story,R.M., Bishop,D.K., Kleckner,N. and Steitz,T.A. (1993) Structural relationship of bacterial RecA proteins to recombination proteins from bacteriophage and yeast. *Science*, **259**, 1892–1896.

Struhl,K. (1995) Yeast transcriptional regulatory mechanisms. *Annu. Rev. Genet.*, **29**, 651–674.

Subramanya,H.S., Bird,L.E., Brannigan,J.A. and Wigley,D.B. (1996) Crystal structure of a DExx box DNA helicase. *Nature*, **384**, 379–383.

Sugawara,N., Ivanov,E.L., Fishman-Lobell,J., Ray,B.L. and Haber,J.E. (1995) DNA structure-dependent requirements for yeast *RAD* genes in gene conversion. *Nature*, **373**, 84–86.

Sung,P. (1994) Catalysis of ATP-dependent homologous DNA pairing and strand exchange by yeast RAD51 protein. *Science*, **265**, 1241–1243.

Sung,P. and Robberson,D.L. (1995) DNA strand exchange mediated by a RAD51–ssDNA nucleoprotein filament with polarity opposite to that of RecA. *Cell*, **82**, 453–461.

Svejstrup,J.Q., Wang,Z., Feaver,W.J., Wu,X., Bushnell,D.A., Donahue,T.F., Friedberg,E.C. and Kornberg,R.D. (1995) Different forms of TFIIH for transcription and DNA repair: holo-TFIIH and a nucleotide excision repairosome. *Cell*, **80**, 21–28.

Szostak,J.W., Orr-Weaver,T.L., Rothstein,R.J. and Stahl,F.W. (1983) The double-strand-break repair model for recombination. *Cell*, **3**, 725–738.

Tabor,S. and Richardson,C.C. (1985) A bacteriophage T7 RNA polymerase/promoter system for controlled exclusive expression of specific genes. *Proc. Natl Acad. Sci. USA*, **82**, 4767–4771.

Wach,A., Brachat,A., Pohlmann,R. and Philippsen,P. (1994) New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae. Yeast*, **10**, 1793–1808.

Weaver,D.T. (1995) What to do at an end: DNA double-strand-break repair. *Trends Genet.*, **11**, 388–392.

West,S.C. (1992) Enzymes and molecular mechanisms of genetic recombination. *Annu. Rev. Biochem.*, **61**, 603–640.

Wood,R.D. (1996) DNA repair in eukaryotes. *Annu. Rev. Biochem.*, **65**, 135–167.

Note added in proof

After submission of the final version of this manuscript the paper by H.Jiang, Y.Xie, P.Houston, K.Stemke-Hall, U.H.Mortensen, R.Rothstein and T.Kodadek (1996) Direct association between the yeast RadS1 and RadS4 recombination proteins. *J. Biol. Chem.*, **271**, 33181–33186, appeared describing also the interaction between RadS1 and RadS4 proteins mediated by the N-terminus of RadS4p.